Copy of
5/8/06 Amendment 4W

Amendments to the Specification:

Please amend the specification as follows:

Please replace paragraph on page 7 starting with "In order to..." with the following rewritten paragraph:

In order to investigate the role of the mammalian *Msh*2 gene in DNA mismatch repair and to address the role of mismatch repair in maintaining genome stability, we generated an ES cell line carrying a disruption in both copies of the mouse *MSH*2 gene. This line is ~~disignated~~ designated dMsh2-9 (ATCC deposit number RH532). Its construction is described in the accompanying manuscript: "Inactivation of the mouse *Msh*2 gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer," by Niels de Wind, Marleen Dekker, Anton Berns, Miroslav Radman, and Hein te Riele, to be published in *Cell*, the 28th July, 1995 on pages 6, 14, 27 (legend to Fig. 1) and 30 (Fig. 1).

3rd Full paragraph on p 7.

copy of p7 of the spec. 9b

destabilization of simple-sequence repeats and an increased rate of recombination between homologous but diverged DNA sequences. The latter phenotype is clearly manifested by the efficient recovery of recombinant bacteria resulting from conjugational crosses between the related but diverged species *Escherichia coli* and *Salmonella typhimurium* wherein the recipient bacterium was deficient for *mutS* or *mutL* (6). Also, the frequency of chromosomal rearrangements by ectopic recombination between diverged sequences is substantially elevated in mismatch repair deficient bacteria (26).

In many respects, the biochemistry of mismatch repair systems in eukaryotes resembles that of the *E. coli mutS,L* system. Homologs of both genes have been identified in yeast and mammalian cells. Based on mismatch binding *in vitro*, and on the mutator and recombinator phenotypes of *Saccharomyces cerevisiae* mutants, the protein encoded by the yeast *MSH2* gene seems to be the functional homolog of MutS (27-29). A homolog of the yeast *MSH2* gene was identified in mammalian cells by analysis of a G·T-mismatch-binding activity, positional cloning and PCR amplification of mouse DNA using degenerate primers (30). Similarly, homologs of the *E. coli mutL* gene were identified in yeast and mammalian cells.

Interestingly, inherited mutations in human *mutS* and *mutL* homologs were recently found to be related to the cancer predisposition syndrome HNPCC (hereditary nonpolyposis colorectal cancer), which is characterized by development of tumors of the proximal colon at early age. In these tumors, mismatch repair is lost, as manifested by destabilization of simple sequence repeats, the replication error-positive (RER^+) phenotype (12).

In order to investigate the role of the mammalian *Msh2* gene in DNA mismatch repair and to address the role of mismatch repair in maintaining genome stability, we generated an ES cell line carrying a disruption in both copies of the mouse *MSH2* gene. This line is ~~designated~~ designated (ATCC Deposit Number RH532) dMsh2-9. Its construction is described in the accompanying manuscript:
"Inactivation of the mouse *Msh2* gene results in mismatch repair deficiency, methylation tolerance, hyperrecombination, and predisposition to cancer", by Niels de Wind, Marleen Dekker, Anton Berns, Miroslav Radman, and Hein te Riele, to be published in *Cell*, the 28th July, 1995 on pages 6, 14, 27 (legend to Fig.1) and 30 (Fig. 1).

As demonstrated in the manuscript, the phenotypic consequences of *Msh2* deficiency in mouse embryonic stem cells provide clear evidence for an essential role of MSH2 in mammalian DNA mismatch repair. First, *Msh2*-deficient ES cells lack binding activity to a double stranded 38-mer oligonucleotide carrying a G·T mismatch or an unpaired TG dinucleotide [pages 6, 15, 27 (legend to Fig.2) and 30 (Fig.2)]. Second, *Msh2*-deficient ES

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